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Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil

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ABSTRACT

Exploiting native plant growth-promoting rhizobacteria (PGPR) in Cameroonian agro-ecosystems provides a means to improve plant–microbe interactions that may enhance ecosystem sustainability and agricultural productivity in an environmentally eco-friendly way. Consequently, we aimed to investigate the community structure and functional PGPR diversity of maize grown in Cameroon. Native bacteria isolated from Cameroon maize rhizosphere soil were identified by partial 16S rRNA gene sequencing and screened for traits particularly relevant for Cameroon low-fertility soil conditions, such as their abilities to tolerate high concentrations of salt, and their plant growth- promoting potential. Genetic and functional diversity was characterized according to their phylogenetic affiliation. A total of 143 bacteria were identified and assigned to 3 phyla (*Actinobacteria*, *Firmicutes* and *Proteobacteria*), 13 families and 20 genera. *Bacillus* (31.5%), *Arthrobacter* (17.5%), and *Sinomonas* (13.3%) were the most abundant genera identified among all the isolates. Based on their *in vitro* characterization, 88.1% were salt tolerant at 2% NaCl, but only 16.8% could tolerate 8% NaCl, 50.4% solubilized phosphate, 10.5% possessed the *nifH* gene, and 19.6% produced siderophores. Six isolates affiliated to the most abundant genera identified in this work, *Bacillus* and *Arthrobacter,* carrying multiple or only single tested traits were selected to evaluate their growth- promoting potential in an *in vitro* maize germination assay. Three strains possessing multiple traits induced significantly increased hypocotyl and root length of maize seeds compared to non-inoculated control seeds. Our results indicate the potential of selected indigenous Cameroon rhizobacteria to enhance maize growth.

1. Introduction

Maize (*Zea mays* L.) is the most widely-grown staple food crop, occupying nearly 17% of the estimated 200 million ha of cultivated land in sub-Saharan Africa. It is also one of the world's three most important food crops (Johnston-Monje and Raizada, 2011), growing under a wide spectrum of soil and climatic conditions around the world (Farooq et al., 2015). In Cameroon, maize is the most consumed cereal, easily exceeding sorghum, rice, or wheat (Manu et al., 2014). Maize is a strategic crop in terms of food security and economic profitability. The plant is mainly grown by small-scale subsistence farmers (Epule and Bryant, 2014). Although maize is cultivated in all five agro-ecological zones of Cameroon and is the most affordable crop in terms of market price and cost of seeds, grain yields often remain low compared with local food demand. One reason is that Cameroonian soils are generally low in fertility, particularly lacking phosphorus (P) and nitrogen (N) (Fankem et al., 2006). Low soil fertility and other issues such as salinity, soil acidity and water stress are the major limiting factors in Cameroonian agriculture, since nutrient deficient and salt-stressed soils are known to severely suppress plant growth and crop productivity. Considering the low corn yields still pervasive in farmers' fields, increasing maize production is an urgent task to secure food supplies in the country.

In particular, the P fixation capacity of soils is a critical problem that

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leads to low soil fertility. P is a component of key molecules such as nucleic acids, phospholipids, and ATP. It is also involved in controlling key enzyme reactions and regulating metabolic pathways, and consequently, plants cannot grow without a reliable supply of this nutrient (Theodorou and Plaxton, 1993). Like many tropical and subtropical soils, Cameroonian soils are predominantly acidic; a high content of iron and aluminum ions effectively react with P in such soils. Consequently, about 75% of P applied as chemical fertilizer or natural rock phosphate is converted into insoluble complexes (Gyaneshwar et al., 2002), making the P-deficiency in soil difficult to overcome.

Seeking a solution, soil microorganisms could contribute efficiently to improving soil fertility. In the rhizosphere, the volume of soil surrounding and under the influence of plant roots is where plants interact with soil microorganisms (Antoun and Prévost, 2005). Many rhizosphere-inhabiting bacterial species are known to exert beneficial effects upon plant growth. Those bacteria are generally referred to as plant growth-promoting rhizobacteria (PGPR). PGPR use various strategies to promote plant growth, such as improving phosphate uptake by solubilizing phosphate complexes into plant absorbable and usable forms, suppressing plant diseases by competitive colonization or inducing systemic or acquired resistance, and producing phytohormones or vitamins (Glick, 2012; Berger et al., 2015). Besides the other plant growth-promoting (PGP) traits, the ability to solubilize different kinds of synthetic inorganic phosphate and natural rock phosphates is particularly crucial in the selection of suitable bacterial candidates for Cameroonian agriculture. Moreover, seeking PGPR possessing novel traits such as salt tolerance will improve salinity management in these nutrient deficient and salt affected soils.

In the rhizosphere of maize grown in many countries, PGP activities have been reported for a series of bacterial species including *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Bacillus* and *Serratia* (Agbodjato et al., 2015; Zahid et al., 2015; Kuan et al., 2016). Despite the potential benefits of using PGPR to enhance crop productivity and improve crop protection under normal and salt stressed conditions (Ahemad and Kibret, 2014; Sharma et al., 2016), these strategies are still largely untapped in the effort to improve maize production in Africa. Especially in Cameroon, little information is available on the occurrence and use of PGP bacteria, and no research has been devoted so far to studying indigenous PGPR associated with maize grown in the country.

It is important to study native microbial communities associated with plants in order to understand their ecological role in specific environments (Cavaglieri et al., 2009). Studies have shown that to maximally exploit the plant-bacteria association effective bacteria must be selected in plant studies that take specific ecological conditions into consideration, e.g. crop management, soil type and climate (Perez-Montano et al., 2014). Under such conditions, knowledge about the native bacterial populations, their identification and their implications for plant physiology, is required for improving management practices regarding plant nutrition and defense.

Application of bacterial inoculants to reduce the use of chemical fertilizers without compromising plant yield and quality is currently an important challenge in agriculture, microbiology, and biotechnology. Towards a sustainable agricultural vision, interest in the beneficial rhizobacteria associated with cereals in particular has increased recently (Vejan et al., 2016). Making this technology readily accessible to farmers in both developed and developing countries and efforts are being made to exploit diverse PGPR as bio-fertilizers for various economically important crops (Ahemad and Kibret, 2014; Zahid et al., 2015). Many PGPR genera and species have been reported to be present in the rhizosphere of numerous crops (Mehnaz et al., 2010). Moreover, they have been used to successfully improve plant growth of maize, rice, wheat, soybean and other horticultural crops both in the laboratory and in the field under various ecological conditions (Shaharoona et al., 2008; Perez-Montano et al., 2014). The use of indigenous PGPR for creating bacterial inoculants can be an advantage since these organisms easily acclimatize to the respective environmental conditions and may more easily establish the plant-microbe interaction (Verma et al., 2013).

In this study, we aim to isolate and characterize rhizobacteria from maize cultivated in the region of Cameroon with the highest maize cropping density. We hypothesize that the rhizosphere of maize grown in Cameroon harbors a high diversity of cultivable bacteria exhibiting multiple plant growth– promoting and salinity tolerance activities. The main goal of our study was to: (i) isolate a wide range of native cultivable bacterial strains from the maize rhizosphere in Cameroon; (ii) characterize the isolates based on their attributes and phylogenic affiliation (partial 16S rRNA gene sequence); (iii) evaluate their *in vitro* potential for salinity tolerance, synthetic and natural inorganic phosphate solubilization, atmospheric nitrogen (N_2) fixation by searching for the presence of *nifH* gene and siderophore production, and (iv) assess the bacterial *in vitro* effect on maize seedlings at the germination stage.

2. Materials and methods

2.1. Study site and sample collection

Soil samples with the characteristics described in Table 1 were collected from maize rhizospheres at a farm in the Ngaoundal locality (6° 30″ North, 13° 16″ East) in the high Guinea savanna zone II, where the southern plateau raises northward to the grassy, rugged Adamawa Plateau (Fig. 1). This feature stretches from the western mountain area and forms a barrier between the country's north and south. Its average elevation is 1,100 m, and temperatures range from 22 °C to 25 °C with high rainfall. The spot was chosen for sample collection because it is the most cultivated maize region in the country. Rhizosphere soils adhering to maize roots at a depth of 10–20 cm were collected from 20 randomized plant rhizospheres of the farm. The samples were mixed to form a composite sample, then packed in a sterile plastic bag and immediately taken to the laboratory. The soil was passed through a 4 mm sieve to eliminate coarse rock and plant material, thoroughly mixed to ensure uniformity, and stored at 4 °C prior to use. A subsample about 0.5 kg was air dried and passed through a 2 mm sieve for chemical analysis. Soil pH was determined in a suspension of soil in saline solutions of neutral reactivity (calcium chloride/$CaCl_2$) in a ratio of 1–2.5 according to Krey et al. (2011). Available amounts of phosphate (P) and potassium (K) were extracted by the double lactate (dl) method and measured using flow injection analysis (FIA) for P (P_{dl}) and atomic absorption spectrophotometry (AAS) for K (K_{dl}) (Krey et al., 2011). Exchangeable magnesium (Mg) was determined using a $CaCl_2$ solution by ASS at 285.2 nm. Total carbon (C_t) and total nitrogen (N_t) contents were measured using a CHN-O Rapid elemental analyzer (Heraeus, Germany) (Ruppel et al., 2006).

2.2. Isolation, purification and conservation of bacterial isolates

The isolation of microorganisms was assessed in non-selective nutrient agar (NA) medium (Standard nutrient agar I, Carl Roth, Germany) containing 6 g NaCl, 3 g yeast extract, 15 g peptone, 1 g glucose, 12 g agar–agar L^{-1}, pH 7. Four independent replicates were analyzed as follows: Ten g soil was homogenized in Erlenmeyer flasks in 90 mL of sterile buffer (NaCl, 0.05 M) by shaking at 290 rpm for one

Table 1
Elemental composition of the soil sample (mean values of four replicates).

pH	mg kg^{-1}					C/N ratio
	P_{dl}	K_{dl}	Mg	N_t	C_t	
5.6	49	62	81	2380	27930	11.7

dl = double lactate extractable